UNPUBLISHED PRELIMINARY DATA

Republic Aviation Corporation
Farmingdale, Long Island, New York
and
SPACE ENVIRONMENT & LIFE SCIENCES LABORATORY

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Quarterly Progress Report (JULY, AUGUST, SEPTEMBER, 1963)
Contract NASw-738)
Study of the Normal Fecal Bacterial Flora of Man

INTRODUCTION

Work on the predominating human fecal flora was started on July 3, 1963. A discussion was held the early part of July with Dr. Frank B. Voris to outline the specific objectives of the first part of this program.

Following this agreed upon schedule, a group of ten healthy, young men were asked to contribute a series of 4-6 fecal samples at approximately monthly intervals over a period of six months. So far two samples at an interval of approximately one month have been obtained from each of these young men. Particular care was taken in the handling of these samples. The samples were collected by the men directly into sterile pint-size ice cream cartons during working hours and were cultured immediately (within fifteen minutes) for the aerobic and anaerobic flora, using procedures as indicated in the contract. The cultures isolated were subjected to further study, including preliminary screen tests.

The other phase of the work conducted in the first quarter of this contract was the study of "type" cultures of the anaerobic organisms. During previous human work in connection with a study on the effect of pure oxygen at various atmospheric pressures on man, several types of cultures had been found repeatedly during anaerobic culturing. These organisms were put through a preliminary series of screen tests so that like organisms could be grouped. Preliminary studies indicated that there were twenty various groups. Work is being done in an attempt to validate these groups.

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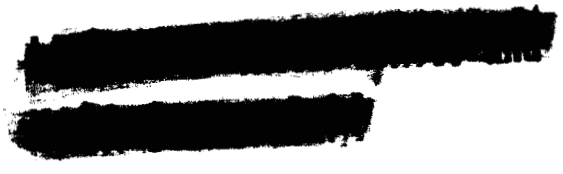
ANAEROBIC CULTURAL SERIES

Two anaerobic cultural series were run on the feces of each of the ten subjects and the results were quite consistent with respect to the number of viable anaerobes that grew in the cultural media. In all but one test out of twenty, the subjects had viable anaerobes in amounts approximating one trillion per gram of feces. Agar shakes were made from the top two or three dilutions of feces showing growth and with only one exception these cultures were strict anaerobes. The one exception occurred in the series that showed the smallest number of viable anaerobes. This aberrant culture was a facultative anaerobe from the lowest dilution which grew (about the 1 billion dilution). These pure cultures were then used for the preliminary screen tests indicated in the contract and the mixed cultures were purified using anaerobic Petri plates. The pure cultures were then screened.

Anaerobic pour plates were also made from the sixth and seventh tubes of the broth series and the colonies were picked from these plates. It has been observed that on primary culture, growth from agar plates would only occur at a lower dilution than in broth culture. The cultures from these colonies were put into agar shakes to determine their degree of anaerobiosis and the pure cultures were screened. The mixed cultures were purified as above and were screened. All but eight of these cultures were obligate anaerobes, six of which were facultative anaerobes, and two were aerobic.

To date, 117 cultures have been screened or are in the process of being screened. Using the "type" culture key which will be described in more detail below, attempts have been made to group the screened cultures. Fourteen cultures were lost because they did not transfer from the agar shake to the screen test media, two cultures were aerobes, and one test was contaminated during screening. One hundred cultures were suitable to attempt "keying". Approximately four-fifths of these cultures appeared to fit into some group in the "key", while one-fifth could not be fitted into any group so far established. These cultures will be studied further. The variety of anaerobic bacteria that could be "keyed out" included almost all of the twenty "established" groups. The variety of bacteria found in any one subject was surprisingly diverse with an average of two out of three cultures in any one subject being different from other cultures in the same subject. Only one subject had a monotonous flora (two "types" out of eight cultures studied).

In comparing the findings of this study with the one carried out on the subjects in the oxygen study where similar techniques were used, it was gratifying to find that a much clearer picture of anaerobiosis was noted on all ten subjects in this study. It is felt that this is a direct result of the rapidity with which the cultures in this study were made from the freshly eliminated feces; something which was not possible on the oxygen study because of lack of easy access to the samples in the chamber. The variety of organisms isolated from each man was greater in the present study



than in the oxygen study. This probably was due to the more rapid culturing and also to the greater variety of food eaten by these subjects as compared to the "canned" diet fed to the men in the oxygen study.

In general, the preliminary results of this anaerobic culturing of human feces to date shows that strict anaerobes are present in approximately one trillion per gram of feces or more. These anaerobes are of diverse types and each man carries a variety of strictly anaerobic organisms.

Gram stains are made on the original 10^{-3} dilution of feces to observe the types of bacteria present in the original sample. Careful observations on these slides reveal, in addition to the usually described bacteria, a predominance of extremely tiny rods and cocci, both gram negative and gram positive. These bacteria would probably be largely obscured in the examination of a slide of the undiluted feces ordinarily observed. The culture of very tiny anaerobic bacteria in the highest dilutions of the feces, coupled with the predominance of such bacteria on the Gram stain, suggest that these tiny bacteria may be an important part of the anaerobic human fecal flora.

As these bacteria are just within the limits of visibility of the microscope, a new microscope with higher powers of resolution and a special light source has been ordered. This microscope should aid in the detection of these microorganisms, both on the Gram stain and in the cultures.

AEROBIC CULTURAL SERIES

One of the important objectives of this study of the predominating fecal flora of normal man is to determine the relative numbers of aerobic and anaerobic bacteria. Therefore, aerobic counting plates were made on each sample. The results obtained from these counting plates showed that less than a billion aerobes were found on each sample. Five subjects had less than one million aerobes per gram of feces and all but two showed less than one hundred million per gram. The other two had roughly 200-550 million aerobes. This shows the striking predominance of the strict anaerobes over the aerobes. Accordingly, emphasis in this contract work will be placed on a study of the anaerobic organisms which predominate in the human fecal flora.

Aerobic studies were conducted on the same samples to determine the general types of aerobes present and to compare the results with descriptions of normal human fecal aerobic flora in the literature. In general, the ten men showed aerobic fecal flora in which the majority of the isolated organisms agreed with what would be expected from descriptions in the literature. However, there were some findings which may be of interest.

1) A coagulase-positive mannitol salt positive staphylococcus was isolated from the feces of one subject on both tests. The subject carried this organism nasally.

2) More Haemophilus was isolated than might have been expected and was particularly prevalent in one subject.

3) The Enterobacteriaceae Providence was isolated on both samples from one individual. Hafnia (37° C) was found on one subject during the first sampling.

4) The variety of different kinds of Escherichia was above expectation and included the isolation of a newly described Escherichia, as well as one β-hemolytic strain.

5) Four types of organisms were found which, according to the literature, might be unusual. These included pneumococci which gave positive results by all recognized tests, Streptococcus bovis and Streptococcus salivarius and Veillonella.

6) Each sample was examined for the presence of potential pathogenic fungi. Three subjects did not show any fungal growth from the fecal sample, while the other seven did. Further work is being done on these cultures.

In general, it can be said that the aerobes are outnumbered by the anaerobes by at least 1000:1 on these ten subjects and that the aerobes found follow, in general, the normal fecal aerobes described in the literature with the few exceptions which are noted above. Because of this, the aerobic work, with the exception of the aerobic counting plate, will be deemphasized during the remainder of the study on these subjects unless some subject shows an unexpectedly marked elevation of the aerobic count.

TYPE CULTURES

During preliminary studies for this contract and the normal fecal flora studies on the men on the oxygen experiment, certain types of anaerobic organisms were isolated repeatedly. An attempt was made to group like organisms and almost 1,000 cultures were put through a short screen test including fermentation of various carbohydrates, litmus milk, and gelatin. The cultures were studied morphologically and for their oxygen requirement in agar shakes. On the basis of these characteristics, about twenty type cultures were described and designated alphabetically.

Since these organisms are difficult to preserve and change characteristics as a result of frequent transfer, lyophilization is being carried out on representative "type" cultures and, at the same time, checks are being run on the stability of the various characteristics used in their designation. So far, five cultures have been "certified" and others are in the process of being studied in this manner. These "certified" "type" cultures will be the basis

for the description of "type" cultures. After these "type cultures have been studied sufficiently for stability, they will be described, photographs will be taken, and then they will be ready for release to any interested scientists.

PROJECT PERSONNEL

Personnel who have been working on the program are Dr. Lorraine S. Gall, Mrs. Phyllis Riely, Mr. Raul Cardenas, Mrs. Shirley Dunwoody, and Mr. Edward Palange.

HOURS EXPENDED (July 3 - September 30)

Professional:	736
Technical:	831

PROJECTED WORK FOR NEXT QUARTER

During the next quarter it is anticipated that the series of samples on the ten healthy, young men who are already under study will be completed and that as many as possible of the "type" cultures will be lyophilized and placed in the "established" group.

Lorraine Gall

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